enzymes in the chemical and pharmaceutical industries. His contribution was wide-ranging in that he considered enzyme reactions using cells, spores, enzymes in solution and, perhaps most interesting of all, immobilised enzymes. Immobilised enzymes have many advantages including increased stability, high specificity, contamination of products is avoided, they can be used many times, reaction volumes can often be decreased and preparation costs are minimised. He discussed in some detail the preparation of immobilised enzymes but stressed that the properties of enzymes can and do change after such a procedure.

Dr. H.U. Bergmeyer and Dr. G. Michal (GFR) confined their attention to the use of enzymes in clinical biochemistry, where enzymes have two main uses, in diagnosis and in therapy, although the latter is extremely limited. From the point of view of diagnosis three developmental trends are evident: i) activity determinations of *new* enzymes and the determination of metabolites with the aid of *new* enzymes; ii) simplification of methods and iii) use of new or improved measuring principles. This last trend was considered in greater detail, and Dr. Bergmeyer discussed the use of immobilised enzymes with a continuous flow as an example of a new principle with great potential.

The last contributer was Dr. P.F. Fox (Ireland) who spoke on the use of enzymes in food processing. Food enzymology can be divided into three well defined areas: i) indigenous food enzymes; ii) added purified enzymes and iii) enzymes produced by microorganisms present either as contaminants or as added cultures.

BIOLOGICAL SYNTHESES

R.B. GILLILARD

Arthur Guiness Son and Co., St. James' Gate, Dublin 8, Ireland

This section of the meeting was divided into three symposia on Brewing, Other Fermentations and Protein Sources.

Biochemists were introduced to some of the technical terminology of brewing by Professor T-M. Enari (Finland) when the addressed them on the subject of 'Malting, Mashing and the use of Unmalted Grain'. He described the mash tun, in which the brewer mashes his malt with warm water and obtains a liquid extract called wort, as a biochemical reactor in which enzymes degrade starch, proteins and other substances to give a nutrient medium suitable for fermentation by yeast. The traditional source of enzymes is malt, which at the same time is the main source of starch in beer production. If malt as a starch source was replaced by cheaper materials such as maize, unmalted barley or wheat, then it would be necessary to replace the malt enzymes by enzymes of microbial origin. As long as malt was used as the main raw material for wort production, said Professor Enari, one could rely on traditional methods of brewing.

The use of unmalted barley was dealt with in some detail and Professor Enari concluded that 25% of the malt used in beer production could be replaced by unmalted barley without any difficulties. The most important factors associated with the brewing quality of the unmalted barley were geographical location of growth and climatic conditions rather than variety of barley.

The 'Chemical Constituents of Hops and their Contribution to Beer' was discussed by Dr. de Keukeleire (Ghent), who must have been encouraged by the number of participants who, at the social functions, were carrying out their own private experiments on beer flavour. The principal bittering components of beer are produced when hops are added to boiling wort. The hop flowers or cones contain the so called alpha acids which are converted, by boiling with wort, into iso-α-acids. As a process, this method of bittering beer is very wasteful and it is usual that only 20–30% of the α-acids originally present in the hop occur as iso-α-acids in the beer. Dr. de Keukeleire then went on to show how iso-α-acids could be modified or degraded to products having an important influence on beer properties such as bittering power and foam stability. The polyphenolic hop fraction, he added, has a definite role in stabilizing beer foam, while free acids behave as anti-gushing agents.

The aroma of hops, he said, is one of the criteria of its quality and derives partly from the essential oil

and partly from the volatile bitter acids oxidation products. Hop oil, like many essential oils, is a complex mixture of hydrocarbon terpenes and their oxidation products. The physical and chemical causes of gushing, the free fatty acids of beer and better understanding of beer aroma were stressed as areas of research which warranted further exploration.

In his lecture on the 'Application of Studies in Yeast Yeast Metabolism to Brewing Science', Professor C.A. Masschelein (Brussels) discussed catabolite repression and inhibition in relation to the utilisation of maltose and maltotriose by brewery yeasts. Maltose and maltotriose are only used at an appreciable rate by yeast after preliminary growth on one of these two sugars. The fermentation is brought about by maltozymase which is composed of two enzymes, a specific maltose permease responsible for the active transport and an α-glucosidase which hydrolyzes the endogeneous maltose or maltotriose. The synthesis and activity of these enzymes, he asserted, are controlled by distinct regulatory processes, particularly repression and catabolic inhibition. He thought that recent results suggested 3′,5′-cyclic-AMP as a pleotropic inducer by simultaneous depression of all systems subject to catabolic repression. As far as brewing yeasts was concerned, he stressed that there is considerable strain variation to these regulatory systems.

The importance of cell wall structure in determining the flocculation characteristics and serological properties of yeasts has been investigated and the speed and regularity of industrial fermentations are linked to the chemical composition of the cell wall.

The study of the transport of amino acids in wort, Professor Masschelein said, has considerable technical importance since the mechanisms responsible for the distribution of intracellular α-amino nitrogen result in common intermediate metabolites which are at the same time precursors of amino acids and higher alcohols.

The symposium on Other Fermentations was started with a lecture on 'Organic Acid Production by Differentiating Fungi' by J.E. Smith (Glasgow) who dealt with the commercial production of citric and itaconic acids and the biochemical reaction mechanisms involved in these processes. At present, he pointed out, most fermentations require several to many days for completion and one of the major aims in fungal organic acid fermentations is to reduce this production time. The time factor delay appears to arise from the well known fact that the fermentation cycle is biphasic – one phase for growth and a second phase for organic acid production. Further, it is by no means clear whether part or all of the fungal biomass is participating in the acid production.

Dr. Smith then went on to discuss the biochemical pathway, not yet clearly defined, by which the hexose substrate is fermented to produce citric acid. All enzymes of the tricarboxylic acid cycle have been shown to be functioning during the fermentation and tightly coupled mitochondria have been isolated at all stages. He suggested that citric acid accumulates during the fermentation as a result of abnormal 3′,5′-cyclic-AMP metabolism leading to loss of glycolytic control. In this way activation of the pathway would produce excess citrate which could then spill over into the medium. However, he offered an alternative explanation in which 3′,5′-cyclic-AMP activates the secretion of citrate from the cell.

The most recent investigations on the biosynthesis of itaconic acid indicate that an important step may be the condensation of three acetate molecules to form 1,2,3-tricarboxypropane which could be decarboxylated to itaconic acid.

What is the present state of our knowledge about the biosynthesis of two important types of fungal peptides, the penicillins and cephalosporins, which contain not only peptide bonds and centres with the D-configuration but also fused and labile ring systems? This was the question posed by Dr. P. Abraham (Oxford) in his contribution entitled 'Studies on the Biosynthesis of Penicillins and Cephalosporins'. The amino acid precursors of the ring systems of these β-lactam antibiotics have been investigated in auxotrophic mutants, It has been demonstrated clearly, Dr. Abraham said, that both ring systems are derived from cysteine, valine and a side-chain precursor in the intact organisms. The O-acetyl group of cephalosporin C is derived from acetate, and the methyl group of 7-methoxy-cephalosporins comes from the methyl group of methionine. The L-isomers of valine and α-aminoadipic acid are more effective precursors than the corresponding D-isomers, although the former give rise to D-configurations in the final products. The origin and fate of the endogenous L-α-aminoadipic acid differs in different types of fermentation. In eukaryotes α-aminoadipic acid is formed from acetyl

coenzyme A and α-oxoglutarate and is mainly used for the synthesis of lysine. In the cephalosporin-producing *Streptomyces* on the other hand, α-amino-adipic acid is formed from lysine and the latter is synthesized, presumably, from diaminopimelic acid. The mechanism of ring closure and the origin of the D-configuration at carbon-3 in the penicillins and in the α-aminoadipyl side-chain of penicillin nitrogen and the cephalosporins were also discussed.

A reappraisal of 'Secondary Metabolism of Micro-organisms' was given by Dr. J. Bu'Lock (Manchester) who defined his subject as the production of secondary metabolites and other metabolic process which are similarly regulated, including such less obvious microbial activities as post-growth lysis, spore formation and toxin production. He concentrated on the basic relationships between growth rate and secondary biosynthesis in *Gibberella fujikuroi* and the more complex system in *Claviceps purpurea* in order to illustrate features of particular significance to a concept of secondary metabolism. He described studies on metabolic differentiation in *G. fujikuroi* using nitrogen as the limiting nutrient supplied as glycine, stressing on interest in the diterpenoid gibberellins and the polyketide pigments, bikaverins. Though these are both formed in response to 'nutrient exhaustion', and have acetyl-CoA as their common precursor, their production is not simultaneous. Similar results are obtained in phosphate-limited cultures. He gave evidence which suggests that in *G. fukikuroi* there is a variety of metabolic sequences specified by 'non-vegetative' genes, which are only expressed in response to different degrees of nutrient limitation such as a common mechanism of 'growth-linked suppression' to which different genes are differently sensitive.

Release from 'growth-linked suppression' as a batch culture develops is shown, in the case of *Claviceps*, to depend critically upon nutritional circumstances and nutrient uptake and growth rate are linked to the level of key metabolic intermediates which in turn must control the suppression effect. In *Claviceps* the suppression over-rides a conventional substrate-induction process.

Dr. F. Moran (London) was the first contributor to the symposium on 'New Protein Sources' when he read a paper prepared by Professor A.V. Champagnat (Marseilles) on 'Proteins from Hydrocarbons'. Dr. Moran spoke of the success that petro-chemical companies were having in making protein foodstuffs from the by-products of petroleum. Ten years ago this subject would have seemed like science fiction but already there were two plants in commercial operation, producing acceptable protein as the result of fermentation reactions with yeasts. The biomass contained more than 60% crude protein before extraction. The production of biomass is most efficient in continuous culture whcih also gives a more reliable and constant quality. The protein has been found to be both nutritious and safe. It contains lower levels of carcinogenic substances than some natural foodstuffs. These 'petrol proteins' have been passed for animal consumption in many European countries and investigations on their use for human consumption were being started. He predicted their use as protein supplement, filling the gap between protein production and world deman for protein.

Professor A. Spicer (High Wycombe) reminded us that the cheapest natural source of protein was still wheat flour when he gave an address on 'The Biosynthesis of Proteins from Carbohydrates'. Economic pressures are forcing the most desirable food items out of the reach of more and more people and with increasing demand for protein foodstuffs he felt sure that no suitable foodstuffs would be made available for rearing cattle because of their relatively inefficient conversion rate. A cow will put on only about 2 kg of protein a day whereas, on the same basis, growing soya beans yielded 50 kg per day; yeasts yielded 50 000 kg and bacteria yielded an astronomical 10^{13} kg per day.

He and his colleagues had chosen carbohydrate as their source of raw material because carbohydrates are a source based on agriculture, a source which can be readily replaced using highly efficient photosynthesis, often under cultural conditions that are unsuitable for other produce. The biomas, resulting from a continuous fermentation with *Fusaria gramineria*, cost roughly the same as wheat flour protein. Its digestibility and biological value compared very favourably with other protein sources and its nutritional value was similar to meat.

He emphasized that it was possible to alter the composition of microbial protein by manipulating the environment in whcih they were produced. In this way it was possible to produce analogues of meat or fish dishes, or completely new foods, which could be obtained without involving very sophisticated and expensive processing.

Dr. G.E. Hall (Sharnbrook) explained why the food industry is developing new foods, which proteins are likely to be used in the future and the technology by which they will be used, when he gave an address on 'Food Proteins of Vegetable Origin'. Dr. Hall referred to the relative inefficiency with which animals convert vegetation to protein and he foresaw that the animals would be by-passed by converting vegetable protein more directly into human foods. Of a long list of potential vegetable protein sources, the most promising were soybeans, cotton seed, leaf, rape seed and the traditional wheat protein.

Soybeans, Dr. Hall said, produce the most protein per acre of any seed crop, the beans containing about 40% protein. Soybeans contain certain antinutritional factors, the best known being the trypsin inhibitor fraction. However, he gave an assurance that this is deactivated and a nutritionally sound protein is obtained by correct processing. The first stage in the isolation of soybean protein involves mechanical expression or liquid extraction of the seed oils to give a flour or meal. The protein is then extracted from the defatted material by dispersion in water at a pH slightly higher than neutrality and insolubles (carbohydrate) are removed before the pH of the extract is adjusted to pH 4.6 (isoelectric point) to precipitate the protein. The isolates are more than 90% protein.

Dr. Hall then went on to describe two general techniques, fibre spinning and thermoplastic extrusion, which have been developed for producing structured soy protein products suitable for meat type products. He showed some colour transparencies to demonstrate the appearance of the final product when served as lumps of dehydrated sponge, brown cubes or shrimp shaped confections, all most effectively garnished and all palatable he assured us. However, as one commentator put it, 'there was certainly nothing that bore any resemblance to a side of beef or a good steak'.

BIOCHEMISTRY AND THE ENVIRONMENT

M. McKILLEN

Department of Biochemistry, Trinity College, Dublin 2, Ireland

In the Symposium on Disposal of Industrial Effluents the first paper was presented by I. Tookos, Budapest, (Hungary). The factors involved in the successful control of organic effluents from food processing industries and the most effective methods available for removal of pollutants from these effluents were described.

Chemical treatment methods, by comparison with mechanical and biological methods, are extremely expensive and less effective. Four treatment procedures and their suitability for dealing with the various food processing effluents were outlined: i) 24 hr aeration of the raw effluent with sludge recirculation, followed by an oxidation lagoon treatment for 2–10 days. This procedure is suitable for the dairy and meat industry, permanently operating canneries and in the starch products industry; ii) plastic-filled trickling filter, followed by chemical coagulation or the use of an aerobic pond. This method is suitable for every branch of the food industry; iii) stabilisation lagoons connected in series with surface aeration are suited to seasonal processing enterprises; iv) plastic-filled trickling filter or anaerobic digestion, followed by activated sludge treatment and finally to an aerobic lagoon, is a method suited to the fermentation industry effluents.

This was followed by C.F. Seyfried (Hanover, G.F.R.) who was concerned with the treatment of organic effluents from non-food industries. The range of effluents to be handled includes those from wood-processing, organic acid fermentation, plastics and mineral oil industries. One feature of these wastes can be their often pronounced toxic properties which must be neutralised before entering potential potable water supplies. Pretreatment should involve elimination of metal ions by ion-exchange, distillation or stripping to remove halogenated hydrocarbons, and various physico-chemical treatments such as hydrolysis to enhance subsequent biodegradation. The biological treatment of choice for different waste waters was outlined.

Then R.K. Chalmers (Birmingham, England) dealt with the treatment of inorganic effluents. The inorganic contaminants resulting from industrial processing range